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Differences between the Sexes in Immune Response and Wound Healing

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Introduction

Chronic wounds are a significant health problem not only in the United States (U.S.), but worldwide. A chronic wound is one that fails to progress within the normal healing trajectory, with the most commonly occurring types of chronic wounds originating from venous ulcers, diabetic foot ulcers, and pressure ulcers. There are nearly 600,000 annual cases of chronic venous ulcers in the U.S., which account for approximately 80% of all chronic leg wounds (Medina, Scott, Ghahary, & Tredget, 2005). The average treatment cost per person for a chronic venous ulcer is about $10,000, which does not account for loss of work productivity (Medina, Scott, Ghahary, & Tredget, 2005). Additionally, foot ulcers develop in approximately 22.5 million diabetic individuals worldwide at least once in their lifetime. Chronic foot ulcers are the most common medical complication in the diabetic populations and contribute to approximately 85,000 lower limb amputations annually (Medina, Scott, Ghahary, & Tredget, 2005). The third most common chronic wound results from nonhealing pressure ulcers, which affect between 1.5 and 3 million people each year (Medina, Scott, Ghahary, & Tredget, 2005).

Due to the pervasiveness of chronic wounds and the substantial cost related to treatment, effective treatment interventions are required. In order to develop effective interventions, molecular and cellular factors that contribute to a chronic wound must first be considered. Since a prolonged inflammatory response has been associated with all chronic wounds, it is logical to look more closely at the mediators that control the initial inflammatory stage of wound healing. The pro-inflammatory cytokines, interleukin-1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) are key mediators of the initial inflammatory response ((Christian, Graham, Padgett, Glaser, & Kiecolt-Glaser, 2006) and some research studies have shown a difference in the production of these pro-inflammatory cytokines between men and women. Furthermore, a

few studies have found that wounds healed more slowly than those of a control group when topical estrogen was applied to a wound bed. Collectively, these findings suggest that the inflammatory response and the wound healing process may be different between men and women.

The majority of the studies that have examined potential sex-related differences in wound healing have used animals, cell lines, or elderly humans, therefore a gap in the literature remains that includes healthy men and women. Specifically, additional studies are needed to clarify whether there is a difference in the local production of pro-inflammatory cytokines in wound fluid between young, healthy men and women and subsequently, a difference in time to complete wound healing. The following analysis will examine data from a primary study (McDaniel, Belury, Ahijevych, & Blakely, 2008) in an attempt to answer two research questions: (1) Is there a difference in local proinflammatory cytokine levels in wound fluid between women and men ages 18 to 45? Hypothesis (1) is that male subjects will have higher levels of pro-inflammatory cytokines in blister fluid when compared to female subjects. The study will also analyze a second question: (2) Is there a difference in the number of days to complete healing between women and men ages 18 to 45? Hypothesis (2) is that male subjects will have a longer number of days to complete healing when compared to female subjects. The knowledge generated from this analysis will provide a foundation for future studies of chronic wounds and help to answer the question of whether the sex of an individual should be considered when choosing treatment interventions.

Review of Literature

Differences in Skin

One of the possible factors that may contribute to wound healing is the thickness of the skin, and in particular the different layers of the skin. Although it is unknown whether the thickness of the skin affects how long it takes for a wound to heal, it is of interest to examine differences in skin for men and women and compare it to wound healing time to determine a possible relationship.

A previous study measured the thickness of the skin in mice and found that the dermal layer was thicker in male mice, and the epidermis and hypodermis were thicker in female mice (Azzi, El-Alfy, Martel, & Labrie, 2004). Toevaluate whether the discovered difference in the thickness of the skin was related to hormones, gonadectomized (GDX) male and female mice were treated for three weeks with 17β-estradiol (E2), dihydrotestosterone (DHT), or dehydroepiandrosterone (DHEA), and then measured again. After treatment with E2, the epidermal thickness increased for both GDX males and GDX females (Azzi, El-Alfy, Martel, & Labrie, 2004), perhaps indicating that epidermal thickness is influenced by estrogen. Treatment with DHEA and DHT resulted in thicker dermal skin for GDX males and GDX females (Azzi, El-Alfy, Martel, & Labrie, 2004), this time showing that dermal thickness may be influenced by the presence of testosterone.

As previously mentioned, the thickness of the skin layers may or may not affect the rate of healing for wounds. However, by demonstrating that hormones have an influence on the thickness of the skin, it becomes of interest to study hormonal influences on wound healing since it is known that hormones have an ability to regulate skin cell growth.

Differences in Time for Wound Healing in Animals

Many studies have observed wound healing in animals, and have experimented altered hormonal levels in these animals. One such study by Ashcroft et al. (1997) found that rats that had been ovariectomized (OVX) had a delayed rate of re-epithelialization, an increase in wound width, and decreased collagen deposition compared to intact rats, perhaps showing that the removal of the source of estrogen impaired wound healing. The study then found that after applying topical estrogen to the wound beds of both OVX and intact animals, the rate of healing was increased, suggesting that applying estrogen, even if the woman still has functioning ovaries, may be beneficial. Many people who have wounds are young and already have circulating estrogen in their bodies, but this study showed that perhaps creating a supraphysiological level of estrogen could be beneficial in wound healing for humans (Ashcroft et al., 1997). Since this study was performed on animals, however, further studies are needed to support the results in humans. Another study conducted by Hardman et al. (2005) found similar results in mice, demonstrating that increased wound areas were apparent in OVX mice when compared to intact mice, further supporting the effect of estrogen on wound healing.

A study by Nitsch et al. (2008) observed immune function in mice following trauma-hemorrhage (T-H). Unlike the previous studies, estrogen was not applied and instead, androgens were depleted in male mice and then wound inflammation and immune cell function were measured. The study found that after depleting the testosterone, inflammation was decreased and immune cell function was increased, resulting in increased wound-breaking strength after injury (Nitsch et al., 2008). These results demonstrate that androgens appear to impair wound healing and increase inflammation, which is opposite of the effects of estrogen. Though more studies are needed and testing would be required in human subjects, this study shows that perhaps men may

have impaired healing not only due to the lack of estrogen but also due to the presence of testosterone.

Differences in Time for Wound Healing in Humans

Many studies have observed the time it takes for wounds to heal in elderly individuals. A study by Ashcroft et al. (1997) found a delayed time for re-epithelialization and reduced matrix collagen deposition in aged women compared to younger women, which suggests that the decrease in estrogen after menopause may contribute to impaired healing. In further support of that hypothesis, the study found that a group of women taking hormone replacement therapy (HRT) after menopause had an accelerated rate of wound healing (Ashcroft et al, 1997). In another study topical estrogen was applied to the wound beds of both elderly men and women. Increased wound healing resulted for those treated with topical estrogen that was evident in wound size and the presence of increased collagen levels for both sexes, with a higher amount of collagen deposition found in females (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999). Furthermore, estrogen treated wounds had increased stiffness that indicates a higher ultimate pressure at breaking point using the nondisruptive dimensional analysis system (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999). This means that the scar was stronger for wounds treated with estrogen. Lastly, the study found that the estrogen treated wound beds had significantly decreased neutrophil numbers which could have implications for chronic wound healing (see Cytokines and Hormones, below). Though this study does not show any major differences between men and women with respect to time for wound healing, it does show the effect of estrogen, a major hormone in women, since the accelerated rate of wound healing was presumably due to the application of estrogen to the wound site.

Collectively, these studies suggest that estrogen increases the rate of wound healing and that estrogen could be used clinically to accelerate wound healing. Estrogen could also be applied topically to the skin before surgery as a preventative intervention, however further studies are needed (Ashcroft et al., 1997). The Ashcroft (1999) study also stated that since a chronic wound is an acute wound which fails to heal, that future research endeavors could test the hypothesis that topical estrogen can accelerate healing in chronic wounds as well as acute wounds (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999).

Pro-Inflammatory Cytokines

Proinflammatory cytokines, mainly produced by circulating neutrophils and macrophages, are an essential part of wound healing. The major proinflammatory cytokines include interleukin-1α (IL-1α), IL-1β, IL-6, IL-8, and TNF-α. They orchestrate various molecular and cellular activities during the wound healing process, such as the remodeling of damaged tissue, which is regulated by fibroblasts and epithelial cells (Christian, Graham, Padgett, Glaser, & Kiecolt-Glaser, 2006). Proinflammatory cytokines also attract phagocytes and other cells that are necessary for tissue regeneration (Kiecolt-Glaser et al., 2005). In acute wounds, neutrophil infiltration and cytokine release is thus, very important for efficient healing; however, a prolonged inflammatory reaction and excessive neutrophil infiltration is associated with chronic, non-healing wounds, which is why chronic wounds also are likely to have elevated interleukin and TNF-α levels (Medina, Scott, Ghahary, & Tredget, 2005).

Cytokine Levels and Hormones

Because of the effect of cytokines on wound healing, it is important to evaluate the potential relationship of cytokines to male and female sex hormones. Many studies have reported on the effects of hormonal regulation on cytokine production, often analyzing the cytokine levels

in response to various alterations in hormone levels. One study found that cultures of cells treated with dehydroepiandrosterone (DHEA), a male sex hormone, produced significantly lower TNF-α, IL-1, and Il-6 than control cultures, perhaps indicating that testosterone decreases cytokine levels, thereby decreasing the inflammatory response (Padgett & Loria, 1998). In another study, inflammation diminished after treatment with dihydrotestosterone (DHT), as measured by edema volume in the joints of rats, again supporting a possible anti-inflammatory effect of testosterone (Ganesan et al., 2008). Furthermore, Nitsch and colleagues (2004) found that intact male mice had suppressed cytokine release following trauma-hemorrhage (T-H), but that depletion of DHT by castration prevented the previously noted depression of IL-1β and IL-6 after T-H. Castration did not, however, affect cytokine release in animals that did not suffer T-H, showing that normal levels of testosterone are only harmful if the host is immunologically compromised (Nitsch et al., 2004). Together these studies support that testosterone may have an anti-inflammatory effect on wounds that may be detrimental to the healing of wounds as cytokine release is essential to wound healing. However, since chronic wounds have a prolonged inflammatory response, it is of interest to evaluate the effects of testosterone on wound healing, as it may decrease the undesired prolonged inflammatory response that causes acute wounds to become chronic wounds.

Numerous studies have also studied the differences between cytokine release in males and females, and the effects of estrogen on cytokine release. One study found that when provoked by the intravenous administration of endotoxin, human females produced more than twice the amount of TNF-α than males, evident in plasma samples obtained at multiple time points after endotoxin administration (van Eijk et al., 2007). Surprisingly, that same study reported that there was no significant difference in the levels of IL-6 for the male and female

cells (van Eijk et al., 2007). Another study found that levels of IL-6 and IL-8 increased when rats underwent ovariectomy (OVX), suggesting that the decrease in estrogen caused an increase in cytokine production (Bruun, Nielsen, Pedersen, Flyvbjerg, & Richelsen, 2003). The study also reported that after being treated with estrogen replacement, the rats then had lower IL-6 and IL-8 levels, demonstrating that estrogen may have an anti-inflammatory effect with regard to cytokine production. Similarly, another study reported that treatment using estrogen reduced the number of local neutrophils, and did so in a dose-dependent manner (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999). Due to this reduction in neutrophil counts, the results suggest that estrogen, again, may have an anti-inflammatory effect and could possibly be used to treat chronic wounds that have prolonged inflammation. One study found conflicting results, however, and reported that 17-β estradiol had no effect on the release of TNF-α or IL-6 from mouse cells (Woodfork, Schuller, & Huffman, 2001). Due to the inconsistent results and the use of mostly animal models, further studies are needed to determine if hormones truly do regulate the release of cytokines and impact wound healing.

The following analysis, therefore, will attempt to answer the question of whether there is a difference in the number of days to complete healing or in levels of pro-inflammatory cytokines in acute wound beds between young healthy females and males.

Methods

Design

The present study is an exploratory secondary analysis of a prospective, randomized, double-blind, repeated measures, experimental study that evaluated the effects of omega-3 fatty acids on wound healing (McDaniel, Belury, Ahijevych, & Blakely, 2008). The outcomes

measured in the primary study were profinflammatory cytokine levels in blister wound fluid, days to complete wound healing, daily area yet to be healed, salivary cortisol and Perceived Stress Scale (PSS) to evaluate the stress response, and plasma fatty acids. The variables of interest in the present study are days to complete wound healing, and proinflammatory cytokine levels in blister wound fluid.

Admission to GCRC (PSS, cortisol)	Blistering	5 hrs (cortisol)	24 hrs (coritsol)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 12-16
R X	Blister Procedure	O1	O1O2	O2	O2	O2	O2	O2	O2	O2	O2
R ~ X	Blister Procedure	O1	O1O2	O2	O2	O2	O2	O2	O2	O2	O2

TABLE KEY X= 4 weeks of omega-EPA and DHA supplements; ~X= 4 weeks of placebo; Blistering= creation of eight 8 mm blisters on nondominant forearm; PSS= perceived stress scale; Cortisol= salivary cortisol; O1= cytokines in blisters; O2= photos of healing

Participants

After Institutional Review Board (IRB) approval was obtained, the sample for the parent study was recruited from The Ohio State University (OSU) academic area and medical center, and included 13 men and 17 women. Inclusion criteria were healthy individuals between 18-45 years of age with the ability to read and write English. Exclusion criteria were individuals taking non-steroidal anti-inflammatory drugs, aspirin, lipid-lowering medications, nutritional supplements, or corticosteroids and those having any immunologic related health problems, chronic inflammatory skin diseases or surgery in the previous year. Additional exclusion criteria included people who were pregnant or lactating, self-reported current smokers, or those reporting drinking 10 or more alcoholic beverages per week.

Measures

Macroscopic analysis of wound healing

Measurement of the daily area yet to be healed was performed using a noncontact method combining single digital camera photogrammetry (SCP) and the Verge Videometer Measurement Documentation (VeV MD) software system. Measurements were made daily for the first 8 days after blister initiation and then every 4 days until 100% wound healing occurred. To measure the area yet to be healed, a card of known dimensions was placed next to the blister sites and photographed. The images were then downloaded to the VeV MD software program and compared to the known size of the card to determine the area, which is explained in detail elsewhere (McDaniel et al., 2008). The area yet to be healed was calculated by the same investigator who was blinded to group assignment (McDaniel et al., 2008).

Proinflammatory cytokine assay

Blister fluid was obtained to measure locally produced proinflammatory cytokines IL-1, IL-6, and TNF-α at 5 and 24 hours post blister formation. Levels were measured using the electrochemilumniscence Multiplex System Sector 2400 imager at the General Clinical Research Center (GCRC) core lab and Human Proinflammatory II 4-Plex Ultra Sensitive Kits (McDaniel et al., 2008). Duplicate assays were performed.

Procedure

The procedure, detailed extensively elsewhere (McDaniel, Belury, Ahijevych, & Blakely, 2008), consisted of recruiting participants from the OSU academic area and medical center. The volunteers who met the inclusion and exclusion criteria, 13 men and 17 women, were randomly assigned to either the EPA/DHA supplement group or placebo group. After informed consent was obtained at the baseline visit, participants in both groups were instructed to take five softgels at night for 4 weeks and then were admitted to the GCRC for a 26-hour stay. During the stay, blisters were formed using a suction blister protocol that included the application of two

templates possessing a total of eight circular orifices, each 8 mm in diameter, to the volar surface of the participant's nondominant arm. A vacuum of 359 mmHg was applied for 1 hour to 90 minutes to form blisters. Fluid was aspirated from each blister using a 27 gauge needle and syringe. The tops of the blisters were removed and two templates that contained a total of 8 wells were places over the blister sites. The blister wells were filled with autologous serum. At 5 hours and 24 hours post blister formation, the fluid was aspirated from the blister wells using an angiocath. Before discharge from the GCRC, standard wound care was initiated. For 8 consecutive days after discharge the blister sites were assessed and measured, and then every four days thereafter until 100% healing occurred. Each participant received $150 for completing the study and parking expenses and meals were provided during the 26-hour stay at the GCRC.

Data Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0. T-tests were performed to compare pro-inflammatory cytokine levels in blister fluid between males and females at 5 and 24 hours post blister formation to address the first hypothesis. T-tests were also used to compare days to complete wound healing between males and females to address the second hypothesis.

Results

Demographic and Nutritional Influences

Statistical analysis of demographic characteristics (see Table 1) revealed that there was no statistically significant difference in age between the groups ($p>.05$). However, the male group had both a significantly higher mean Body Mass Index (BMI) ($t=2.308$, $df=28$, $p=.029$) and mean Sagittal Abdominal Diameter (SAD) ($t=2.175$, $df=28$, $p=.038$), compared to the female

group which could potentially influence wound healing because the adipocytes contained in adipose tissue can synthesize both tumor necrosis factor-α (TNF-α) and several interleukins (IL), notably IL-1β and IL-6 (Power & Schulkin, 2008).

Proinflammatory Cytokine Responses

Proinflammatory cytokines IL-1, IL-6, and TNF-α were measured in the blister fluid of all participants at 5 hours and 24 hours post-blister formation. As expected, the mean blister fluid levels of TNF- α were significantly higher in the male group than in the female group at both 5 hours ($t=2.332$, $df=28$, $p<.05$) and 24 hours ($t=2.479$, $df=25$, $p<.05$) post blister formation. Mean levels of IL-1 and IL-6 were also higher in the blister fluid of the male group at both time points when compared to the female groups, but the differences were not statistically significant.

Wound Healing Measures

Time to complete wound healing was significantly different between the two groups, revealing that the male group required a significantly longer number of days (mean= 12 days) than the female group (mean= 9 days) to achieve 100% healing. A regression analysis of all primary study variables (McDaniel, Belury, Ahijevych, & Blakely, 2008) revealed that male gender alone explained 38% of the variance in number of days to complete healing.

Discussion

In the present secondary analysis study, male sex was associated with a significantly higher number of days to complete wound healing, levels of the proinflammatory cytokine TNF-α in the blister fluid of acute wounds, and SAD measures as compared to the female group. With respect to the number of days to complete healing, the study results are aligned with the current hypothesis as well as findings from previous studies. One such study found that a group taking

hormone replacement therapy (HRT) after menopause had an accelerated rate of wound healing (Ashcroft et al, 1997), indicating that estrogen may improve and hasten wound healing. In another study, estrogen was applied topically to the wound beds of both elderly men and women (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999). There was increased wound healing, with respect to wound size and collagen levels in the skin of both sexes with a higher amount of collagen deposition found in females (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999). The findings suggest that estrogen enhances wound healing. The results of the current study are consistent with these previous studies because we know that young, healthy females naturally have higher levels of estrogen than males, and we found that the males indeed took a significantly higher number of days for complete healing than the females.

The differences in proinflammatory cytokine levels in blister fluid were also as hypothesized for the present study, with respect to TNF- α, with significantly higher mean levels in the male group compared to the female group. These results are consistent with several previous studies which concluded that estrogen may exert an anti-inflammatory effect in the healing process. A study by Bruun and colleagues (2003) found that circulating levels of IL-6 increased when rats underwent ovariectomy (OVX), suggesting that the decrease in estrogen caused an increase in cytokine production. If the lack of estrogen leads to higher cytokine levels, then it is logical to hypothesize that an increase in estrogen would be associated with a decrease in cytokine production. The results of the current study support this idea because the female participants, who likely have higher levels of estrogen than the males, had significantly lower levels of the proinflammatory cytokine TNF- α. However, the study by Bruun et al. (2003) measured only systemically produced levels of IL-6 and IL-8, whereas the current study

analyzed IL-1, IL-6, and TNF- α levels that were secreted locally in an acute wound. Hence, the results are only indirectly comparable.

In a related study, scientists reported that the topical application of estrogen reduced the number of local neutrophils in wounds, and did so in a dose-dependent manner (Ashcroft, Greenwell-Wild, Horan, Wahl, & Ferguson, 1999). Though this study by Ashcroft et al. did not address the proinflammatory cytokines that were of interest in the current study, the results support the notion that estrogen may have anti-inflammatory actions because an increased production of neutrophils is associated with a heightened inflammatory response.

Though our finding that male sex was associated with higher levels of the proinflammatory cytokine TNF-α in wound fluid agrees with the results of a few previous studies, it also contrasts with the results of several other studies. As mentioned above, one study found that cultures of cells treated with DHEA produced significantly lower TNF-α, IL-1, and Il-6 than control cultures (Padgett & Loria, 1998), which is not consistent with our finding that males had higher levels of proinflammatory cytokines. Our results also differ from the results of a study by van Eijk et al. (2007), which reported that human females produced more than twice the amount of TNF-α than males, which is different from our finding that females produced significantly lower amounts of TNF-α than the males. These contradicting findings may be because Eijk et al. (2007) measured systemic levels of proinflammatory cytokines, while our study measured the local production of proinflammatory cytokines in response to acute wounding. Another explanation for the opposing results is that the mean age of the females in the study by Eijk et al. (2007) was lower than the mean age of the females in the present study.

IL-1 levels in the blister fluid were not significantly higher in males in the present study, which was consistent with the results of the study by Padgett and Loria (1998) mentioned above.

However, we predicted that IL-1 levels would be significantly higher because it was assumed that if TNF-α and IL-6 were higher, that IL-1 would be higher as well. Perhaps a study employing a larger sample size would find significant differences in wound fluid levels of IL-1.

IL-6 levels were also non-significantly different between the two groups in the current study, which is consistent with the results of a previous study that reported that there was no significant difference in the levels of IL-6 produced by males and females when provoked by intravenous endotoxin administration (van Eijk et al., 2007). The study by Bruun et al. (2003), however, found that after an ovariectomy (and hence the removal of estrogen) in mice increased systemic levels of IL-6 and that hormone replacement subsequently lowered levels of IL-6. Therefore, with regard to proinflammatory cytokines, the findings of the current study do not clarify the contradicting findings of previous studies.

An interesting secondary finding in the current study revealed that the mean SAD of the male group was significantly higher than the female group. A higher SAD may also have influenced levels of proinflammatory cytokines and days to complete healing because SAD is a measurement of visceral fat stores (Power & Schulkin, 2008). Higher levels of visceral fat are associated with higher levels of IL-6 (Smith, 2008), therefore it is possible that the male group in the current study had higher levels of proinflammatory cytokines in blister fluid than the female group because they also had more visceral fat. Additionally, the male group had a higher amount of total body fat than the female group as reflected by a significantly higher mean BMI. Males have been found to have naturally higher levels of visceral fat than females even though females are more likely to have higher levels of subcutaneous fat (Power & Schulkin, 2008). Since visceral fat may influence the production and storage of proinflammatory cytokines, it is unknown whether the results of this study are due to the impact of visceral fat or hormonal

regulation of proinflammatory cytokine release. It is therefore important that future studies that are designed to observe potential differences in wound healing between the sexes carefully consider these variables.

There are several limitations of the current study. Most importantly, it is a secondary analysis of a larger study, therefore the target variables of our study were not the target variables of the primary study. Because the goal of this study was to explore the primary study findings, it was not possible to add a measurement of hormone levels in the study participants. Measures of sex hormones would have added another important dimension to our findings. The omega-3 fatty acid supplement intervention of the parent study may have influenced wound healing and the inflammatory response, however we were able to statistically control for this variable. Lastly, because the mean SAD measures were found to be significantly different between males and females in the current study analysis, and it is known that visceral fat influences proinflammatory cytokine production, the findings cannot be solely attributed to the sex of the participants. Therefore the results of the current study must be considered in light of these limitations.

In summary, since the current study, along with previous studies suggests that estrogen enhances the rate of healing, perhaps estrogen could be used clinically to accelerate wound healing. It could also be applied topically to skin before surgery as an intervention to improve wound healing and prevent the development of chronic wounds. The knowledge generated from the present study also suggests that sex-specific wound assessments and interventions may be beneficial, but additional research is needed. The present study results may guide future research endeavors that observe the relationships between wound healing and the sex of the client. Furthermore, findings from acute wound research may be applied to chronic wound research in

order to eventually develop interventions that will improve the healing of both acute and chronic wounds.

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Table 1.

Demographic and Nutritional Characteristics of Participants

	Female	Male	P-value
Age, mean years (SD)	25(6.2)	26(6.6)	0.441
*BMI, mean kilograms/meter^2 (SD)	23.9(4.5)	28.9(7.4)	0.029
*SAD, mean cm (SD)	17.8(3.0)	21.1(5.2)	0.038

BMI= body mass index SAD= sagittal abdominal diameter

*denotes significant difference between groups